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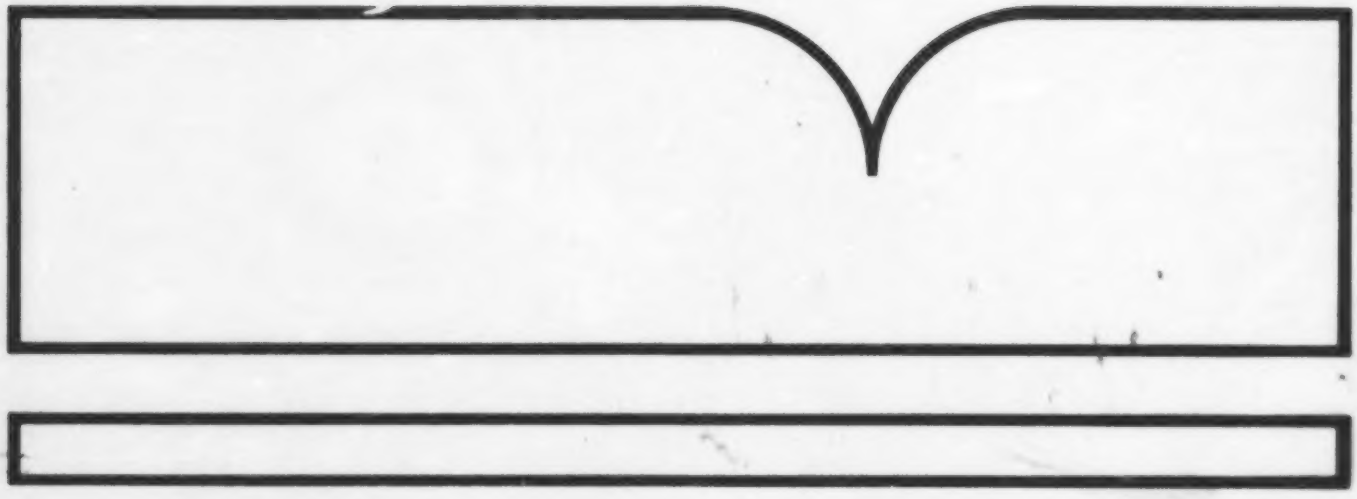

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Observations of Rat Fetuses after Irradiation with 2450-MHz (CW) Microwaves*

E. Berman[1], H.B. Carter[1] and D. House[2]

ABSTRACT

Female Sprague-Dawley (CD) rats were exposed to 2450-MHz (CW) microwave radiation at incident power densities of 0 or 28 mW/cm² for 100 min daily on the 6th through 15th day of gestation. The whole-body specific absorption rate at 28 mW/cm² is estimated to be 4.2 W/kg. These exposure conditions raised rats' average colonic temperatures to 40.3°C at the end of irradiation. There were 67 sham-irradiated and 70 microwave-irradiated females. When these groups were compared, no significant differences were found in pregnancy rates; in the numbers of live, dead, or total fetuses; in the incidences of external, visceral, or skeletal anomalies or variations; or in the body weight of live fetuses. It is concluded that these conditions do not have an effect on the gross structure of the fetal rat when applied repetitively during post-implantation pregnancy. It is also strongly suspected that this lack of an effect may hold true at any exposure level less than that which will kill a significant number of the dams by hyperthermia (colonic temperature > 40°C).

INTRODUCTION

A previous report from our laboratory dealt with observations of fetal mice exposed to 2450-MHz microwaves [1]. In that report, there was evidence that microwaves can cause alterations in the mouse fetus without inducing a severe general temperature response in the dam. Investigators that have used the rat have concentrated their efforts on experiments in which dams' temperatures exceeded 40°C, and have been able to demonstrate a number of teratologic alterations in the fetus that are related to the exposure regimen [2–10]. We report here an attempt to explore the fetotoxic potential of microwaves in the rat under circumstances similar to that conducted in mice. This study is part of a series of experiments covering a range of frequencies, power densities, and species of laboratory animals.

MATERIALS AND METHODS

All the animals used in this study were CD* (Sprague-Dawley) 90-day-old female rats. Groups of 16 virgin animals were time-bred by the supplier (the day that a copulatory plug was seen was considered day 1) and were received in our animal facility on day 3 of gestation. Nine such groups were bred and received, one group per week. Each group of 16 was assigned randomly to subgroups of 8 microwave-irradiated and 8 sham-irradiated rats. Treatment (microwave-irradiation or sham-irradiation) began on day 6 and continued daily through day 15. Each treatment was for 100 min and occurred between 1000 and 1500 h daily.

On each day of treatment the animals were placed in individual Plexiglass containers, 18 cm long x 10 cm high x 11 cm base-width and 6 cm top-width, on foamed polystyrene trays** to catch body wastes (Fig. 1). The containers were positioned in an area 70 cm x 55 cm, with the long axes of the containers parallel to the H-field vector. The animals occupied the same position each day of treatment.

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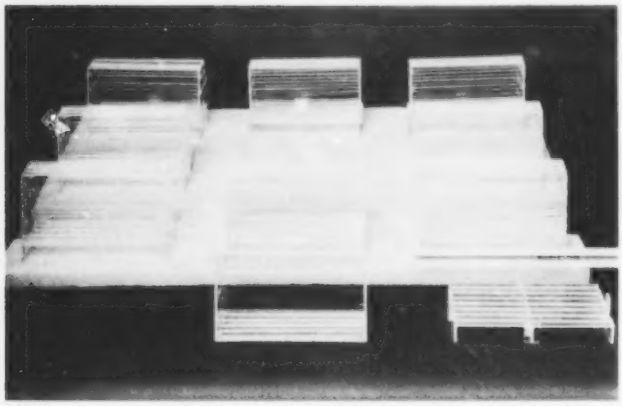

Figure 1 Containers used for rats in this experiment and their positions in the microwave field. For display purposes, one additional cage is separated and placed at the lower border. The ruler measures in inches and is parallel to the H-vector.

Irradiation was conducted in a 2450 MHz (CW) anechoic facility [11,12]. The incident power density was 28 mW/cm² applied to the dorsum of the subjects. Ambient temperature was 22.2 ± 1°C, relative humidity was 50 ± 5%, and air flow was 0.08 - 0.1 m/s. Sham-irradiated subjects were treated identically, but without radiation, in a chamber adjacent to the anechoic chamber; the air flow was common to both groups of animals. Other than periods of treatment, the subjects were maintained in shoe-box type cages in which feed and water were available *ad lib*.

On the 21st day of pregnancy, each female was killed, its uterus exposed and examined for pregnancy, and the number of live, dead, and resorbed conceptuses tallied. Each live fetus was blotted dry, examined for external morphology, weighed, and fixed either in Bouin's fixative or in ethyl alcohol fixative (every 3rd living fetus). At a later date, razor-sections were made of the head, and the abdomen and thorax were incised ventrally to allow examination of internal morphology of the Bouin's-fixed fetuses. Alcohol-fixed fetuses were cleared for skeletal visualization (KOH-Alizarin) and examined under 15X magnification for morphology of ribs and sternebrae.

Frequency-type data (pregnancy and anomaly rates) were expressed as litter units and were analyzed by experimental treatment using Chi-Square tests. The individual fetal values for rib and sternebrae numbers were grouped by treatment before analysis as frequency-type data. Continuous data (numbers of live, dead and resorbed, and total fetuses, and live fetal weight) were expressed by litter and analyzed using analysis of variance.

Colonic temperatures of six naive rats (350–430 g) were measured immediately before and after irradiation at 28 mW/cm² for 100 min. Temperatures were determined with a digital electronic thermometer using a thermistor probe.

RESULTS

There were no significant differences of any variable examined in this study between microwave-irradiated and sham-irradiated animals. The pregnancy rates (microwave-irradiated: 64/67 = 96%; sham-irradiated: 66/70 = 94%) were similar in both groups (Table 1) and appear to be normal. Mean litter values for live, dead and resorbed, and total fetuses, and live fetal weight were similar in both groups and appear to be normal (because only one dead fetus was seen, "dead" was added to the "resorbed" fetus category). The numbers and incidences of ribs and sternal ossification centers in the sham-irradiated and microwave-irradiated groups were similar (Table 2) and appear normal. The types and the incidences of the types of major and minor terata were similar in both groups of litters. Skeletal and visceral

Table 1 Results of exposure of CD rats to 0 or 28 mW/cm², 2450-MHz (CW) microwaves for 100 min daily, days 6 through 15 of pregnancy.
[1]Values are means ± 1 SD.

Power Density (mW/cm²)	0	28	X²	df	F	df
Bred animals	67	70				
Pregnant animals	64	66				
% Pregnant	96	94	0.11	1		
Live fetuses/litter[1]	10.6 ± 3.5	9.9 ± 3.2			1.43	1
Dead and resorbed fetuses/litter[1]	0.9 ± 1.1	0.8 ± 1.0			0.56	1
Total fetuses/litter[1]	11.5 ± 3.4	10.7 ± 3.2			1.84	1
Mean live fetal weight (g)/litter[1]	3.99 ±0.44	3.95 ± 0.42			0.16	1

Table 2 Results of skeletal examination of fetuses of CD rats exposed to 0 or 28 mW/cm², 2450-MHz (CW) microwaves for 100 min daily, days 6 through 15 of pregnancy.
[1]Values are means ± 1 SD.

Power Density (mW/cm²)	0	28
Number of fetuses	215	207
Number of ribs[1]	13.0 ±0.1	13.0 ± 0.1
Number of sternebrae[1]	5.6 ± 0.6	5.6 ±0.6
Number of fetuses with:		
12 ribs	1	0
13 ribs	214	206
14 ribs	0	1
Number of fetuses with:		
3 sternebrae	3	2
4 sternebrae	7	11
5 sternebrae	59	54

variations of minor nature had similar incidences in both groups. No encephaloceles were seen in any of these litters. Mean and standard deviation values for colonic temperatures of the six rats was 38.2 ± 0.5°C before and 40.3 ± 0.4°C after irradiation with a power density of 28 mW/cm² for 100 min.

DISCUSSION

Major anomalies in the Sprague-Dawley strain of rat appear in less than 0.5% of living fetuses [13,14]. It is against this background of low incidence that we attempted to discern morphologic changes induced by microwave exposure. A low rate of spontaneous malformation might be expected to aid the detection of teratogenesis due to a test substance [14″, 15″, page 163]. The rat can offer another advantage when used in teratologic studies of microwaves. As the E-vector to H-vector ratio of specific absorption rate (SAR) of the rat is near unity at 2450 MHz [16], permitting free movement during exposure does not exceptionally confound dose-controlled experiments. Also, by using large samples (67 sham-irradiated and 70 microwave-irradiated dams), we expected to enhance the detection of anomalies and avoid the problems associated with the interpretation of chance occurrences in small populations.

This study was intended to be a dose-response study. We began at a power density of 28 mW/cm² which, under our conditions, will raise the rat's mean colonic temperature to 40.3°C. The estimated SAR is approximately 4.2 W/kg [17, page 96]. It was expected that if a significant increase in teratologic incidence was associated with irradiation at 28 mW/cm², then lower power densities would be investigated.

Colonic temperatures much in excess of 40°C are symptomatic of hyperthermia. Such temperatures represent limits to the experiments conducted in our laboratory. The interest which we have in the fetotoxic potential of microwaves is below the range of exposures which cause frank hyperthermia. Because incident power densities much higher than 28 mW/cm² would have caused frank hyperthermia in the dams, further study was discontinued.

There is a considerable number of reports of attempts to elicit teratologic responses in rodents as a result of exposure to radiofrequency electromagnetic radiation. There are at least two reviews of the available pertinent literature in the mouse and rat [1,18].

It is known that frank hyperthermia can result in teratologic response. Such a response has been shown for high ambient temperatures [19], for intense radiofrequency radiation [2–10] and for intense infrared radiations [6]. But, the teratogenic response when maternal temperatures are $<41°C$ has been difficult to elicit. Dietzel [10], for instance, saw no increase in morphologic abnormalities (tail and extremities) in rat fetuses when irradiation conditions produced a mean colonic temperature below 41°C in the dam. Jensh, *et al.* did not find significant differences between control rat fetuses and those exposed to "non-thermal" levels of 2450-MHz [7] or 915-MHz microwaves [8]. Chernovetz, *et al.* [9] also did not find significant morphologic alterations in rat fetuses exposed at 2450-MHz microwaves, even though the mean colonic temperature reached 40.6°C. Another report by these authors [10] strongly suggests that only those acute-exposure regimens that cause the dam's colonic temperature to exceed 40°C result in teratogenicity in the rat fetus.

The determination of the public health risk of radio-frequency radiation exposure may rest on published scientific reports. All of the studies so far reported have used mice or rats as the experimental subject. The appropriateness of the mouse and the rat as suitable animal models for human fetotoxic effects of radio-frequency radiation therefore deserves close attention.

One must make the initial assumption that the human fetus can be responsive to radio-frequency radiation in exposure situations which are not lethal for the mother. Exposure situations in which fetotoxicity is dependent only upon maternal lethality are not of interest to the public health as a fetal risk. A model which requires a significant loss of the maternal population in which to show any fetal effects is not a practical model based on the assumption above. Another model would estimate the public health risk by demonstrating fetotoxicity when there is no actual risk to humans. In the public health view, this last is an over-estimation, but is more appropriate than the first, even if the assumption is wrong.

The fetal rat appears to lack the capability to respond to radio-frequency radiation by a clear demonstration of effects at doses less than that which are lethal to a portion of the dams. The lowest, but effective, acute dose for teratogenesis in the rat is apt to cause maternal physiologic extremes, including death. This is the over-riding theme of published reports of the attempts to induce fetal changes in the rat using radio-frequency radiation. Until results of studies not yet published show otherwise, we can only conclude that the rat is an inappropriate model for such human fetal risk. The mouse, on the other hand, does have the capability to demonstrate fetotoxic effects at radio-frequency radiation doses which are not also a maternal hazard. In experiments where decreased fetal weight of mice was a significant result of exposure to 2450-MHz microwaves, the dams' mean body temperature was less than 40°C [1]. The mouse fetus can also respond to doses of radio-frequency radiation which kill a significant fraction of the dams. (A review of the literature on the fetotoxic effects of microwaves in the mouse is found in Ref. [1]). The mouse fetus, then, appears to grade its response to graded doses. These types of characteristics point to the mouse fetus as an appropriate model for human risk.

In summary, daily exposures of pregnant rats to microwaves at an estimated SAR of 4.2 W/kg is not fetotoxic. We also conclude that microwave radiation at levels that cause no significant general maternal thermal response in the rat do not have a strong teratogenic activity in that species.

REFERENCES

1 Berman, E., J.B. Kinn, and H.B. Carter, Observations of Mouse Fetuses After Irradiation with 2.45-GHz Microwaves, Health Physics 35:791–801, 1978.

2 Dietzel, F. and W. Kern, Abortion Following Ultra-Short-Wave Hyperthermia: Animal Experiments, Arch. F. Gynak. 209:237–255, 1970.

3 Dietzel, F., W. Kern, and R. Steckenmesser, Deformity and Intrauterine Death After Short-Wave Therapy in Early Pregnancy in Experimental Animals, Munch. Med. Wochensch. 114:228–230, 1972.
4 Dietzel, F., Effects of Electromagnetic Radiation on Implantation and Intrauterine Development of the Rat, N.Y. Acad. Sci. 247:367–376, 1975.
5 Laskey, J., D. Dawes, and M. Howes, Progress Report on 2450 MHz Irradiation of Pregnant Rats and the Effect on the Fetus, *in* Radiation Bio-Effects Summary Report, PHS, USDHEW, BRH/DBE-70, Rockville, MD, pp. 167–173, 1970.
6 Chernovetz, M.E., D.R. Justesen, and A.F. Oke, A Teratological Study of the Rat: Microwave and Infrared Radiations Compared, Radio Science 12(6):191–197, 1977.
7 Jensh, R.P., J. Ludlow, I. Weinberg, W.H. Vogel, T. Rudder, and R.L. Brent, Studies Concerning the Effects of Protracted Prenatal Exposure to a Non-Thermal Level of 2450 MHz Microwave Radiation in the Pregnant Rat, Teratology 17(2):48A, 1978.
8 Jensh, R.P., J. Ludlow, I. Weinberg, W.H. Vogel, T. Rudder, and R.L. Brent, Teratogenic Effects on Rat Offspring of Non-Thermal Chronic Prenatal Microwave Irradiation, Teratology 15(2):14A, 1977.
9 Chernovetz, M.E., D.R. Justesen, N.W. King, and J.E. Wagner, Teratology, Survival and Reversal Learning After Fetal Irradiation by 2450 MHz Microwave Energy, J. Microwave Power 10(4):391–409, 1975.
10 Chernovetz, M.E., D.R. Justesen, and D.M. Levinson, Acceleration and Deceleration of Fetal Growth of Rats by 2450-MHz Microwave Irradiation, *in* Electromagnetic Fields in Biological Systems, S.S. Stuchly, editor, IMPI, Canada, pages 175–193, 1979.
11 Elder, J.A. and J.S. Ali, The Effect of Microwaves (2450 MHz) on Isolated Rat Liver Mitochondria, *in* Biologic Effects of Nonionizing Radiation, P.E. Tyler, editor, Ann. N.Y. Acad. Sci. 247:251–262, 1975.
12 Blackman, C.F., S.G. Benane, C.M. Weil, and J.S. Ali, Effects of Nonionizing Electromagnetic Radiation on Single-Cell Biologic Systems, ibid, pages 352–366.
13 Palmer, A.K., Sporadic Malformations in Laboratory Animals and Their Influence on Drug Testing, *in* Drugs and Fetal Development, M.A. Klingberg, A. Abramovici, and J. Chemke, editors, Adv. Exp. Med. Biol. 27:45–60, 1972.
14 Perraud, J., Levels of Spontaneous Malformations in the CD Rat and CD-1 Mouse, Lab. Anim. Sci. 26(2):293–300, 1976.
15 Wilson, J.G., Environment and Birth Defects, Academic Press, NY, 1973.
16 Gage, M.I., E. Berman, and J.B. Kinn, Videotape Observation of Rats and Mice During Exposures to 2450 MHz Microwave Radiation, Radio Science 14(6S):227–232, 1979.
17 Durney, C.H., C.C. Johnson, P.W. Barber, H. Massoudi, M.F. Iskander, J.L. Lords, D.K. Ryser, S.J. Allen, and J.C. Mitchell, Radiofrequency Radiation Dosimetry Handbook, 2nd Edition, SAM-TR-78-22, USAF School of Aerospace Medicine, Brooks AFB, TX, 1978.
18 O'Connor, M.E., Mammalian Teratogenesis and Radio-Frequency Fields, Proc. IEEE 68(1):56–60, 1980.
19 Edwards, M.J. and R.A. Wanner, Extremes of Temperature, Chapter 12, pages 421–444, *in* Handbook of Teratology, J.G. Wilson and F.C. Fraser, editors, Vol. 1, Plenum Press, NY, USA, 1977.